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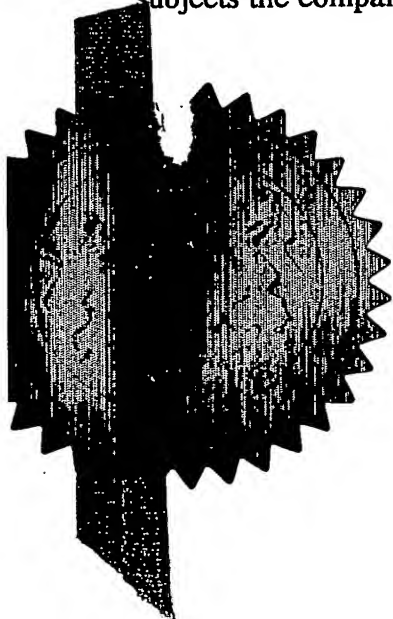
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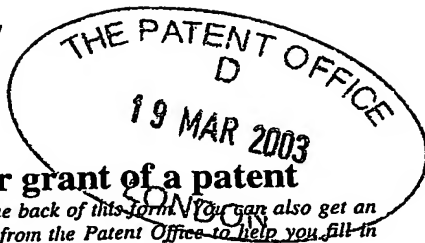
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20MAR03 E793565-1 D01030  
P01/7700 0.00-0306309.6

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**0306309.6**

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473587003.

Patents ADP number (if you know it)

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4 Title of the invention

**METHOD OF TREATMENT**

5 Name of your agent (if you know one)

**STEPHANIE A. LEAROYD**

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## Method of Treatment

### Field of the Invention

The present invention relates to a method of treatment of neurological diseases. In particular the invention relates to the use of anti-MAG antibodies to promote oligodendrocyte survival in a human, particularly following an oxidative stress event.

### Background of the Invention

Myelin-associated glycoprotein is a cell surface transmembrane molecule expressed on the surface of myelin consisting of five extracellular immunoglobulin domains, a single transmembrane domain and an intracellular domain. MAG expression is restricted to myelinating glia: oligodendrocytes in the central nervous system and Schwann cells in the peripheral nervous system. MAG interacts with neuronal receptor(s) which initiates bi-directional signalling between neurons and glia. The effect of MAG binding to neuronal receptors is known to result in inhibition of neurite outgrowth in vitro. Based on this in vitro data, antagonists of MAG have been postulated as useful for the promotion of axonal sprouting following injury (WO9522344, WO9701352 and WO9707810), although these claims are not supported by *in vivo* data. WO02/062383 discloses that an anti-MAG antibody, when administered directly into the brain or intravenously following focal cerebral ischaemia in the rat (a model of stroke) provides neuroprotection and enhances functional recovery.

Evidence in the literature suggests that MAG also mediates signalling into glial cells, but the functional significance of this has not been understood. It has been reported that engagement of MAG expressed at the surface of a CHO cell using an antibody leads to the activation of fyn kinase (Umemori et al 1994, Nature, 367, 572-576). Furthermore, MAG knockout animals exhibit defects in myelin which resemble aspects of the defective changes observed in the brains of multiple sclerosis and encephalomyelitis patients (Lassman et al., Glia, 19, 104-110).

It has now been found that an anti-MAG monoclonal antibody previously shown to provide benefit in a rat model of stroke protects oligodendrocytes from oxidative-induced cell death in vitro. Furthermore, an increased number of histologically intact oligodendrocytes were observed in the brains of rats treated with anti-MAG compared to control antibody following middle-cerebral artery occlusion. Therefore anti-MAG antibodies (or MAG antagonists) may provide dual activities of promotion of neuronal regeneration as well as surprisingly triggering pathways which promote oligodendrocyte survival.

Oligodendrocyte damage or degeneration is observed in several neurological diseases including Alzheimer's disease (e.g. Roher et al, 2002, Biochemistry 41:11080-90; Xu et al,

2001, J Neurosci. 2001 21:RC118), spinal cord injury (e.g. Crowe et al,1997, Nature Medicine, 1997, 3:73-6; Beattie et al, 2002, Neuron 36:375-86), traumatic brain injury (e.g. Castejon et al, 2000, Brain Inj. 2000 Apr;14(4):303-17) and multiple sclerosis. Therefore anti-MAG antibody or antagonists could provide benefit in these diseases in addition to stroke by both promoting neuronal regeneration and preventing oligodendrocyte cell death.

### **Brief Summary of the Invention**

In one aspect, the present invention provides a method of promoting oligodendrocyte survival in a human suffering or at risk of developing a neurological disease which comprises administering to said human a therapeutically effective amount of an anti-MAG antibody, including altered antibodies or a functional fragment thereof.

In another aspect, the invention provides the use of an anti-MAG antibody, including altered antibodies or a functional fragment thereof in the preparation of a medicament for the promotion of oligodendrocyte survival in a human suffering or at risk of developing a neurological disease.

Other aspects and advantages of the present invention are described further in the detailed description and the preferred embodiments thereof.

### **Description of the figures**

Figure 1: Anti-MAG monoclonal antibody protects oligodendrocytes from glutamate mediated cell death.

Figure 2: Oligodendrocytes present in ischaemic lesion with and without anti-MAG antibody.

Figure 3: Anti-MAG chimera binding to rat MAG.

### **Detailed Description on the Invention**

Neurological diseases which may be treated by the method of the present invention include stroke, traumatic brain injury and spinal cord injury as well as chronic diseases including Alzheimer's disease, fronto-temporal dementias (tauopathies), peripheral neuropathy, Parkinson's disease, Huntington's disease and multiple sclerosis. Anti-MAG mabs therefore may be useful in the treatment of these diseases as well as any other disorders related to oxidative stress and/or the degeneration of myelin or oligodendrocytes.

It has now been found that an anti-MAG monoclonal antibody, described (Poltorak et al (1987) *Journal of Cell Biology* 105,1893-1899, DeBellard et al (1996) *Mol. Cell. Neurosci.* 7, 89-101; Tang et al (1997) *Mol. Cell. Neurosci.* 9, 333-346; Torigoe K and Lundborg G (1997)

*Exp. Neurology* 150, 254-262) and commercially available (MAB1567 (Chemicon)) when administered either directly into the brain or intravenously following focal cerebral ischaemia in the rat (a model of stroke) directly protects oligodendrocytes from oxidative induced cell death. This antibody is a murine antibody. Although murine antibodies are often used as diagnostic agents their utility as a therapeutic has been proven in only a few cases. Their limited application is in part due to the repeated administration of murine monoclonals to humans usually elicits human immune responses against these molecules. To overcome these intrinsic undesirable properties of murine monoclonals "altered" antibodies designed to incorporate regions of human antibodies have been developed and are well established in the art. For example, a humanised antibody contains complementarity determining regions ("CDR's") of non human origin and the majority of the rest of the structure is derived from a human antibody.

The antibody useful in the invention is preferably a fully human antibody or an altered antibody, preferably a monoclonal antibody (mAb) and is more preferably chimeric, humanised or reshaped, more preferably humanised.

The human or altered antibody preferably has the structure of a natural antibody or fragment thereof. The antibody may therefore comprise a complete antibody, a (Fab<sup>1</sup>)<sub>2</sub> fragment, a Fab fragment, a light chain dimer or a heavy chain dimer. The antibody may be an IgG1, IgG2, IgG3, or IgG4; or IgM; IgA, IgE or IgD or a modified variant thereof. The constant domain of the antibody heavy chain may be selected accordingly. The light chain constant domain may be a kappa or lambda constant domain.

The constant region is selected according to the functionality required. Normally an IgG1 will demonstrate lytic ability through binding to complement and will mediate ADCC (antibody dependent cell cytotoxicity). An IgG4 will be preferred if a non-cytotoxic blocking antibody is required. However, IgG4 antibodies can demonstrate instability in production and therefore it may be more preferable to modify the generally more stable IgG1. Suggested modifications are described in EPO307434, preferred modifications include at positions 235 and 237. The invention therefore provides a lytic or a non-lytic form of an antibody according to the invention.

In a preferred aspect the altered antibody useful in the present invention is class IgG, more preferably IgG1.

Preferably the antibody or functional fragment thereof useful in the present invention binds to MAG and comprises one or more of the following CDR's. The CDR's are identified as described by Kabat (Kabat et al. (1991) Sequences of proteins of immunological interest; Fifth

Edition; US Department of Health and Human Services; NIH publication No 91-3242. CDRs preferably are as defined by Kabat but following the principles of protein structure and folding as defined by Chothia and Lesk, (Chothia et al., (1989) Conformations of immunoglobulin hypervariable regions; Nature 342, p877-883) it will be appreciated that additional residues may also be considered to be part of the antigen binding region and are thus encompassed by the present invention.

### ***Light chain CDRs***

<b><i>CDR</i></b>	<b><i>According to Kabat</i></b>
L1	KSSHSVLYSSNQKNYLA (Seq ID No 1)
L2	WASTRES (Seq ID No 2)
L3	HQYLSSLT Seq ID No 3)

### ***Heavy chain CDRs***

<b><i>CDR</i></b>	<b><i>According to Kabat</i></b>
H1	NYGMN (Seq ID No 4)
H2	WINTYTGEPTYADDFTG (Seq ID No 5)
H3	NPINYYGINYEGYVMDY (Seq ID No 6)

The present invention also relates to the use of an antibody which binds to the same epitope as an antibody having the CDRs described above. Competitive inhibition assays are used for mapping of the epitopes on an antigen.

More preferably, the altered antibody or functional fragment thereof useful in the present invention comprises a heavy chain variable domain which comprises one or more CDR's selected from CDRH1, CDRH2 and CDRH3 and for a light chain variable domain which comprises one or more CDRs selected from CDRL1, CDRL2 and CDRL3.

Most preferably the altered anti-Mag antibody or functional fragment thereof useful in the present invention comprises:

- a) a heavy chain variable domain ( $V_H$ ) which comprises in sequence CDRH1, CDRH2 and CDRH3,  
and /or

b) a light chain variable domain ( $V_L$ ) which comprises in sequence CDRL1, CDRL2 and CDRL3

5 As discussed above, altered antibodies include chimeric antibodies which comprise variable regions deriving from one species linked to constant regions from other species. Chimeric mouse-human anti-MAG immunoglobulin light and heavy chains useful in the present invention are provided below:

10 Sequence of a mouse/human chimeric anti-MAG antibody heavy chain in which the murine anti-MAG heavy chain variable region is associated with a functional immunoglobulin secretion signal sequence, and with an altered form of the human IgG1 constant region, in which Kabat residues 248 and 250 have been mutated to alanine in order to disable the  
 15 G. Localization of the C1q binding site on antibodies by surface scanning. Nature 332, 738-740, 1988. Duncan, A.R., Woolf, J.M., Partridge, L.J., Burton, D.R. and Winter, G.

MGWSCIIILFLVATATGVHSEIQLVQSGPELKKPGETNKISCKASGYTFTNYGMNWWVKQAPGKGLKW  
 MGWINTYTGEPTYADDFTRFAFSLETSASTAYLQISNLKNEDTATYFCARNPINYYGINYEGYVM  
 20 DYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF  
 PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELAG  
 APSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV  
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL  
 VKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN  
 25 HYTQKSLSLSPGK (Seq ID No 7)

Sequence of a mouse/human chimeric anti-MAG antibody light chain in which the murine anti-MAG light chain variable region is associated with a functional immunoglobulin secretion signal sequence, and with the human kappa constant region.

30 MGWSCIIILFLVATATGVHSNIMMTQSPSSSLAVSAGEKVTMSCKSSHSVLYSSNQKNYLAWYQQKPG  
 QSPKLLIYWASTRESGVPRFTGSGSGTDFTLTIIIVHTEDLAVYYCHQYLSSLTFTGTGKLEIKR  
 TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS  
 LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (Seq ID No 8)

35 Sequence of a mouse/human chimeric anti-MAG antibody heavy chain which provides the amino acid sequence of a chimeric immunoglobulin heavy chain in which the murine anti-



MAG heavy chain variable region is associated with a functional immunoglobulin secretion signal sequence, and with a wild-type type form of the human IgG1 constant region.

5 MGWSCIILFLVATATGVHSEIQLVQSGPELKKPGETNKISCKASGYTFTNYGMNWVKQAPGKGLKW  
 DYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF  
 PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG  
 GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV  
 10 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL  
 VKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHN  
 HYTQKSLSLSPGK (Seq ID No 9)

15 Thus for example the present invention includes an altered antibody comprising a heavy chain Sequence ID No 9 or 7 and/or a light chain Seq ID No 8.

20 Mouse-human chimeras using human 1gG1, 1gG2, 1gG3, 1gG4, 1GA, 1gE, 1gM, 1gD constant regions may be produced, as may chimeras associating the mouse variable regions with heavy or light chain constant regions from non-human species.

Most preferably, the antibody for use in the present invention is humanised antibody or functional fragment thereof that binds to one of MAG and comprises a heavy chain variable region comprising the following amino acid sequences:

25 QVQLVQSGSELKKPGASVKVSCASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTGEPT  
YADDFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCARNPINYYGINYEGYVMDYWGQGTL  
 VTVSS (Seq ID No 10)

30 QVQLVQSGSELKKPGASVKVSCASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTGEPT  
YADDFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCARNPINYYGINYEGYVMDYWGQGTL  
 VTVSS (Seq ID No 11)

35 QVQLVQSGSELKKPGASVKVSCASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTGEPT  
YADDFTGRFVFSLDTSVSTAYLQISSLKAEDTATYFCARNPINYYGINYEGYVMDYWGQGTL  
 VTVSS (Seq ID No 12)

QVQLVQSGSELKKPGASNKVSCASGYTFTNYGMNWVRQAPGQGLEWMGWINTYTGEPT  
YADDFTGRFVFSLDTSVSTAYLQISSLKAEDTATYFCARNPINYYGINYEGYVMDYWGQGTL  
 VTVSS (Seq ID No 13)

In each of these cases each of the 4 heavy chains are preferably combined with one of 4 light chain variable regions :

5 DIVMTQSPDSLAVSLGERATINCKKSSHVLYSSNQKNYLAWYQQKPGQPPKLLIYWASTRE  
SGVPDRFSGSGSGTDFTLTISLQAEDVAVYYCHQYLSSLTFGQGGTKLEIKRTV (Seq ID No  
 14)

10 DIVMTQSPDSLAVSLGERATINCKKSSHVLYSSNQKNYLAWYQQKPGQPPKLLIYWASTRE  
SGVPDRFSGSGSGTDFTLTIIINLQAEDVAVYYCHQYLSSLTFGQGGTKLEIKRTV (Seq ID No  
 15)

15 DIVMTQSPDSLAVSLGERATINCKKSSHVLYSSNQKNYLAWYQQKPGQPPKLLIYWASTRE  
SGVPDRFSGSGSGTDFTLTISLHTEDVAVYYCHQYLSSLTFGQGGTKLEIKRTV (Seq ID No  
 16)

20 DIVMTQSPDSLAVSLGERATINCKKSSHVLYSSNQKNYLAWYQQKPGQPPKLLIYWASTRE  
SGVPDRFSGSGSGTDFTLTIIINLHTEDVAVYYCHQYLSSLTFGQGGTKLEIKRTV (Seq ID No  
 17)

25 Preferred antibodies for use in the present invention therefore include all combinations of preferred elements listed above. In particular the invention includes all combinations of each of the above 4 heavy chains (Seq ID No 10 – 13) with each of the 4 light chains (Seq ID No 14-17).

25 "Neutralising" refers to substantial inhibition of MAG function including its binding to neurones and inhibition of neurite outgrowth.

30 "Substantial inhibition" refers to 75%, more preferably 85%, most preferably 95% inhibition measured in *in vitro* tests.

35 "Altered antibody" refers to a protein encoded by an altered immunoglobulin coding region, which may be obtained by expression in a selected host cell. Such altered antibodies include engineered antibodies (e.g., chimeric, reshaped, humanized or vectored antibodies) or antibody fragments lacking all or part of an immunoglobulin constant region, e.g., Fv, Fab, or F(ab)<sub>2</sub> and the like.

"Altered immunoglobulin coding region" refers to a nucleic acid sequence encoding altered antibody. When the altered antibody is a CDR-grafted or humanized antibody, the sequences

that encode the complementarity determining regions (CDRs) from a non-human immunoglobulin are inserted into a first immunoglobulin partner comprising human variable framework sequences. Optionally, the first immunoglobulin partner is operatively linked to a second immunoglobulin partner.

"First immunoglobulin partner" refers to a nucleic acid sequence encoding a human framework or human immunoglobulin variable region in which the native (or naturally-occurring) CDR-encoding regions are replaced by the CDR-encoding regions of a donor antibody. The human variable region can be an immunoglobulin heavy chain, a light chain (or both chains), an analog or functional fragments thereof. Such CDR regions, located within the variable region of antibodies (immunoglobulins) can be determined by known methods in the art. For example Kabat et al. (Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987)) disclose rules for locating CDRs. In addition, computer programs are known which are useful for identifying CDR regions/structures.

"Second immunoglobulin partner" refers to another nucleotide sequence encoding a protein or peptide to which the first immunoglobulin partner is fused in frame or by means of an optional conventional linker sequence (i.e., operatively linked). Preferably it is an immunoglobulin gene. The second immunoglobulin partner may include a nucleic acid sequence encoding the entire constant region for the same (i.e., homologous - the first and second altered antibodies are derived from the same source) or an additional (i.e., heterologous) antibody of interest. It may be an immunoglobulin heavy chain or light chain (or both chains as part of a single polypeptide). The second immunoglobulin partner is not limited to a particular immunoglobulin class or isotype. In addition, the second immunoglobulin partner may comprise part of an immunoglobulin constant region, such as found in a Fab, or F(ab)<sub>2</sub> (i.e., a discrete part of an appropriate human constant region or framework region). Such second immunoglobulin partner may also comprise a sequence encoding an integral membrane protein exposed on the outer surface of a host cell, e.g., as part of a phage display library, or a sequence encoding a protein for analytical or diagnostic detection, e.g., horseradish peroxidase,  $\beta$ -galactosidase, etc.

The terms Fv, Fc, Fd, Fab, or F(ab)<sub>2</sub> are used with their standard meanings (see, e.g., Harlow et al., Antibodies A Laboratory Manual, Cold Spring Harbor Laboratory, (1988)).

As used herein, an "engineered antibody" describes a type of altered antibody, i.e., a full-length synthetic antibody (e.g., a chimeric, reshaped or humanized antibody as opposed to an antibody fragment) in which a portion of the light and/or heavy chain variable domains of a selected acceptor antibody are replaced by analogous parts from one or more donor

antibodies which have specificity for the selected epitope. For example, such molecules may include antibodies characterized by a humanized heavy chain associated with an unmodified light chain (or chimeric light chain), or vice versa. Engineered antibodies may also be characterized by alteration of the nucleic acid sequences encoding the acceptor antibody light and/or heavy variable domain framework regions in order to retain donor antibody binding specificity. These antibodies can comprise replacement of one or more CDRs (preferably all) from the acceptor antibody with CDRs from a donor antibody described herein.

A "chimeric antibody" refers to a type of engineered antibody which contains a naturally-occurring variable region (light chain and heavy chains) derived from a donor antibody in association with light and heavy chain constant regions derived from an acceptor antibody.

A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one (or more) human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity (see, e.g., Queen et al., Proc. Natl Acad Sci USA, 86:10029-10032 (1989), Hodgson et al., Bio/Technology, 9:421 (1991)).

"Reshaped human antibody" refers to an altered antibody in which minimally at least one CDR from a first human monoclonal donor antibody is substituted for a CDR in a second human acceptor antibody. Preferably all six CDRs are replaced. More preferably an entire antigen combining region (e.g., Fv, Fab or F(ab')<sub>2</sub>) from a first human donor monoclonal antibody is substituted for the corresponding region in a second human acceptor monoclonal antibody. Most preferably the Fab region from a first human donor is operatively linked to the appropriate constant regions of a second human acceptor antibody to form a full length monoclonal antibody.

A "vectored antibody" refers to an antibody to which an agent has been attached to improve transport through the blood brain barrier (BBB). The attachment may be chemical or alternatively the moiety can be engineered into the antibody. One example is to make a chimera with an antibody directed towards a brain capillary endothelial cell receptor eg an anti-insulin receptor antibody or anti-transferrin receptor antibody (Saito et al (1995) *Proc. Natl. Acad. Sci. USA* 92 10227-31; Pardridge et al (1995) *Pharm. Res.* 12 807-816; Broadwell et al (1996) *Exp. Neurol.* 142 47-65; Bickel et al (1993) *Proc Natl. Acad. Sci. USA* 90, 2618-2622; Friden et al (1996) *J. Pharm. Exp. Ther.* 278 1491-1498, US5182107, US5154924, US5833988, US5527527). Once bound to the receptor, both components of the bispecific antibody pass across the BBB by the process of transcytosis. Alternatively the agent may be a ligand which binds such cell surface receptors eg insulin, transferrin or low density lipoprotein (Descamps et al (1996) *Am. J. Physiol.* 270 H1149-H1158; Duffy et al (1987) *Brain*

*Res.* 420 32-38; Dehouck et al (1997) *J. Cell Biol.* 1997 877-889). Naturally occurring peptides such as penetratin and SynB1 and Syn B3 which are known to improve transport across the BBB can also be used (Rouselle et al (2000) *Mol. Pharm.* 57, 679-686 and Rouselle et al (2001) *Journal of Pharmacology and Experimental Therapeutics* 296, 124-131).

5

The term "donor antibody" refers to an antibody (monoclonal, or recombinant) which contributes the amino acid sequences of its variable regions, CDRs, or other functional fragments or analogs thereof to a first immunoglobulin partner, so as to provide the altered immunoglobulin coding region and resulting expressed altered antibody with the antigenic specificity and neutralizing activity characteristic of the donor antibody.

10

The term "acceptor antibody" refers to an antibody (monoclonal, or recombinant) heterologous to the donor antibody, which contributes all (or any portion, but preferably all) of the amino acid sequences encoding its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to the first immunoglobulin partner. Preferably a human antibody is the acceptor antibody.

15

"CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains. See, e.g., Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987). There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, "CDRs" as used herein refers to all three heavy chain CDRs, or all three light chain CDRs (or both all heavy and all light chain CDRs, if appropriate). The structure and protein folding of the antibody may mean that other residues are considered part of the antigen binding region and would be understood to be so by a skilled person. See for example Chothia et al., (1989) Conformations of immunoglobulin hypervariable regions; *Nature* 342, p877-883.

20

25

CDRs provide the majority of contact residues for the binding of the antibody to the antigen or epitope. CDRs of interest in this invention are derived from donor antibody variable heavy and light chain sequences, and include analogs of the naturally occurring CDRs, which analogs also share or retain the same antigen binding specificity and/or neutralizing ability as the donor antibody from which they were derived.

30

35

A "functional fragment" is a partial heavy or light chain variable sequence (e.g., minor deletions at the amino or carboxy terminus of the immunoglobulin variable region) which retains the same antigen binding specificity and/or neutralizing ability as the antibody from which the fragment was derived.

An "analog" is an amino acid sequence modified by at least one amino acid, wherein said modification can be chemical or a substitution or a rearrangement of a few amino acids (i.e., no more than 10), which modification permits the amino acid sequence to retain the biological characteristics, e.g., antigen specificity and high affinity, of the unmodified sequence. For example, (silent) mutations can be constructed, via substitutions, when certain endonuclease restriction sites are created within or surrounding CDR-encoding regions. The present invention contemplates the use of analogs of the antibody of the invention. It is well known that minor changes in amino acid or nucleic acid sequences may lead eg to an allelic form of the original protein which retains substantially similar properties. Thus analogs of the antibody of the invention includes those in which the CDRs in the hypervariable region of the heavy and light chains are at least 80% homologous, preferably at least 90 % homologous and more preferably at least 95 % homologous to the CDRs as defined above as CDRH1, CDRH2, CDRH3, CDRL1, CDRL2 and CDRL3 and retain MAG neutralising activity. Amino acid sequences are at least 80% homologous if they have 80% identical amino acid residues in a like position when the sequences are aligned optimally, gaps or insertions being counted as non-identical residues.

Analogues may also arise as allelic variations. An "allelic variation or modification" is an alteration in the nucleic acid sequence. Such variations or modifications may be due to degeneracy in the genetic code or may be deliberately engineered to provide desired characteristics. These variations or modifications may or may not result in alterations in any encoded amino acid sequence.

The term "effector agents" refers to non-protein carrier molecules to which the altered antibodies, and/or natural or synthetic light or heavy chains of the donor antibody or other fragments of the donor antibody may be associated by conventional means. Such non-protein carriers can include conventional carriers used in the diagnostic field, e.g., polystyrene or other plastic beads, polysaccharides, e.g., as used in the BIAcore [Pharmacia] system, or other non-protein substances useful in the medical field and safe for administration to humans and animals. Other effector agents may include a macrocycle, for chelating a heavy metal atom, or radioisotopes. Such effector agents may also be useful to increase the half-life of the altered antibodies, e.g., polyethylene glycol.

A neutralising antibody specific for MAG has been described (Poltorak et al (1987) *Journal of Cell Biology* 105,1893-1899, DeBellard et al (1996) *Mol. Cell. Neurosci.* 7, 89-101; Tang et al (1997) *Mol. Cell. Neurosci.* 9, 333-346; Torigoe K and Lundborg G (1997) *Exp. Neurology* 150, 254-262) and is commercially available (MAB1567 (Chemicon)).

Alternatively, one can construct antibodies, altered antibodies and fragments, by immunizing a non-human species (for example, bovine, ovine, monkey, chicken, rodent (e.g., murine and rat), etc.) to generate a desirable immunoglobulin upon presentation with native MAG from any species against which antibodies cross reactive with human MAG can be generated, eg human or chicken. Conventional hybridoma techniques are employed to provide a hybridoma cell line secreting a non-human mAb to MAG. Such hybridomas are then screened for binding using MAG coated to 384- or 96-well plates, with biotinylated MAG bound to a streptavidin coated plate. or in a homogenous europium-APC linked immunoassay using biotinylated MAG.

A native human antibody can be produced in a human antibody mouse such as the "Xenomouse" (Abgenix) where the mouse immunoglobulin genes have been removed and genes encoding the human immunoglobulins have been inserted into the mouse chromosome. The mice are immunised as normal and develop an antibody reponse that is derived from the human genes. Thus the mouse produces human antibodies obviating the need to humanize the after selection of positive hybridomas. (See Green L.L., *J Immunol Methods* 1999 Dec 10;231(1-2):11-23).

The present invention also includes the use of Fab fragments or F(ab')<sub>2</sub> fragments derived from mAbs directed against MAG. These fragments are useful as agents protective *in vivo*. A Fab fragment contains the entire light chain and amino terminal portion of the heavy chain; and an F(ab')<sub>2</sub> fragment is the fragment formed by two Fab fragments bound by disulfide bonds. Fab fragments and F(ab')<sub>2</sub> fragments can be obtained by conventional means, e.g., cleavage of mAb with the appropriate proteolytic enzymes, papain and/or pepsin, or by recombinant methods. The Fab and F(ab')<sub>2</sub> fragments are useful themselves as therapeutic or prophylactic, and as donors of sequences including the variable regions and CDR sequences useful in the formation of recombinant or humanized antibodies as described herein.

The Fab and F(ab')<sub>2</sub> fragments can also be constructed via a combinatorial phage library (see, e.g., Winter et al., *Ann. Rev. Immunol.*, 12:433-455 (1994)) or via immunoglobulin chain shuffling (see, e.g., Marks et al., *Bio/Technology*, 10:779-783 (1992), which are both hereby incorporated by reference in their entirety.

Thus human antibody fragments (Fv, scFv, Fab) specific for MAG can be isolated using human antibody fragment phage display libraries. A library of bacteriophage particles, which display the human antibody fragment proteins, are panned against the MAG protein. Those phage displaying antibody fragments that bind the MAG are retained from the library and clonally amplified. The human antibody genes are then excised from the specific

bacteriophage and inserted into human IgG expression constructs containing the human IgG constant regions to form the intact human IgG molecule with the variable regions from the isolated bacteriophage specific for MAG.

- 5 The donor antibodies may contribute sequences, such as variable heavy and/or light chain peptide sequences, framework sequences, CDR sequences, functional fragments, and analogs thereof, and the nucleic acid sequences encoding them, useful in designing and obtaining various altered antibodies which are characterized by the antigen binding specificity of the donor antibody.

10 Taking into account the degeneracy of the genetic code, various coding sequences may be constructed which encode the variable heavy and light chain amino acid sequences, and CDR sequences as well as functional fragments and analogs thereof which share the antigen specificity of the donor antibody. Isolated nucleic acid sequences, or fragments thereof,  
15 encoding the variable chain peptide sequences or CDRs can be used to produce altered antibodies, e.g., chimeric or humanized antibodies, or other engineered antibodies when operatively combined with a second immunoglobulin partner.

20 Altered immunoglobulin molecules can encode altered antibodies which include engineered antibodies such as chimeric antibodies and humanized antibodies. A desired altered immunoglobulin coding region contains CDR-encoding regions that encode peptides having the antigen specificity of an anti-MAG antibody, preferably a high affinity antibody, inserted into a first immunoglobulin partner (a human framework or human immunoglobulin variable region).

25 Preferably, the first immunoglobulin partner is operatively linked to a second immunoglobulin partner. The second immunoglobulin partner is defined above, and may include a sequence encoding a second antibody region of interest, for example an Fc region. Second immunoglobulin partners may also include sequences encoding another immunoglobulin to  
30 which the light or heavy chain constant region is fused in frame or by means of a linker sequence. Engineered antibodies directed against functional fragments or analogs of MAG may be designed to elicit enhanced binding.

35 The second immunoglobulin partner may also be associated with effector agents as defined above, including non-protein carrier molecules, to which the second immunoglobulin partner may be operatively linked by conventional means.

Fusion or linkage between the second immunoglobulin partners, e.g., antibody sequences, and the effector agent may be by any suitable means, e.g., by conventional covalent or ionic



bonds, protein fusions, or hetero-bifunctional cross-linkers, e.g., carbodiimide, glutaraldehyde, and the like. Such techniques are known in the art and readily described in conventional chemistry and biochemistry texts.

5     Additionally, conventional linker sequences which simply provide for a desired amount of space between the second immunoglobulin partner and the effector agent may also be constructed into the altered immunoglobulin coding region. The design of such linkers is well known to those of skill in the art.

10    In still a further embodiment, the antibody of the invention may have attached to it an additional agent. For example, the procedure of recombinant DNA technology may be used to produce an engineered antibody of the invention in which the Fc fragment or CH<sub>2</sub>-CH<sub>3</sub> domain of a complete antibody molecule has been replaced by an enzyme or other detectable molecule (i.e., a polypeptide effector or reporter molecule).

15    The second immunoglobulin partner may also be operatively linked to a non-immunoglobulin peptide, protein or fragment thereof heterologous to the CDR-containing sequence having the antigen specificity of anti-MAG antibody. The resulting protein may exhibit both anti-MAG antigen specificity and characteristics of the non-immunoglobulin upon expression. That  
20    fusion partner characteristic may be, e.g., a functional characteristic such as another binding or receptor domain, or a therapeutic characteristic if the fusion partner is itself a therapeutic protein, or additional antigenic characteristics.

25    Another desirable protein of this invention may comprise a complete antibody molecule, having full length heavy and light chains, or any discrete fragment thereof, such as the Fab or F(ab')<sub>2</sub> fragments, a heavy chain dimer, or any minimal recombinant fragments thereof such as an F<sub>V</sub> or a single-chain antibody (SCA) or any other molecule with the same specificity as the selected donor mAb. Such protein may be used in the form of an altered antibody, or may be used in its unfused form.

30    Whenever the second immunoglobulin partner is derived from an antibody different from the donor antibody, e.g., any isotype or class of immunoglobulin framework or constant regions, an engineered antibody results. Engineered antibodies can comprise immunoglobulin (Ig) constant regions and variable framework regions from one source, e.g., the acceptor  
35    antibody, and one or more (preferably all) CDRs from the donor antibody. In addition, alterations, e.g., deletions, substitutions, or additions, of the acceptor mAb light and/or heavy variable domain framework region at the nucleic acid or amino acid levels, or the donor CDR regions may be made in order to retain donor antibody antigen binding specificity.

Such engineered antibodies are designed to employ one (or both) of the variable heavy and/or light chains of the anti-MAG mAb or one or more of the heavy or light chain CDRs. The engineered antibodies may be neutralising, as above defined.

- 5 Such engineered antibodies may include a humanized antibody containing the framework regions of a selected human immunoglobulin or subtype, or a chimeric antibody containing the human heavy and light chain constant regions fused to the anti-MAG antibody functional fragments. A suitable human (or other animal) acceptor antibody may be one selected from a conventional database, e.g., the KABAT® database, Los Alamos database, and Swiss  
10 Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain constant region and/or a heavy chain variable framework region for insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain constant or variable framework  
15 regions may be selected in a similar manner. It should be noted that the acceptor antibody heavy and light chains are not required to originate from the same acceptor antibody.

Desirably the heterologous framework and constant regions are selected from human immunoglobulin classes and isotypes, such as IgG (subtypes 1 through 4), IgM, IgA, and IgE.  
20 However, the acceptor antibody need not comprise only human immunoglobulin protein sequences. For instance a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding a non-immunoglobulin amino acid sequence such as a polypeptide effector or reporter molecule.

- 25 Preferably, in a humanized antibody, the variable domains in both human heavy and light chains have been engineered by one or more CDR replacements. It is possible to use all six CDRs, or various combinations of less than the six CDRs. Preferably all six CDRs are replaced. It is possible to replace the CDRs only in the human heavy chain, using as light chain the unmodified light chain from the human acceptor antibody. Alternatively, a  
30 compatible light chain may be selected from another human antibody by recourse to the conventional antibody databases. The remainder of the engineered antibody may be derived from any suitable acceptor human immunoglobulin.

The engineered humanized antibody thus preferably has the structure of a natural human  
35 antibody or a fragment thereof, and possesses the combination of properties required for effective therapeutic use.

It will be understood by those skilled in the art that an engineered antibody may be further modified by changes in variable domain amino acids without necessarily affecting the

specificity and high affinity of the donor antibody (i.e., an analog). It is anticipated that heavy and light chain amino acids may be substituted by other amino acids either in the variable domain frameworks or CDRs or both.

- 5 In addition, the constant region may be altered to enhance or decrease selective properties of the molecules of the instant invention. For example, dimerization, binding to Fc receptors, or the ability to bind and activate complement (see, e.g., Angal et al., Mol. Immunol., 30:105-108 (1993), Xu et al., J. Biol. Chem., 269:3469-3474 (1994), Winter et al., EP 307,434-B).
- 10 An altered antibody which is a chimeric antibody differs from the humanized antibodies described above by providing the entire non-human donor antibody heavy chain and light chain variable regions, including framework regions, in association with immunoglobulin constant regions from other species, preferably human for both chains.
- 15 Preferably, the variable light and/or heavy chain sequences and the CDRs of suitable donor mAbs, and their encoding nucleic acid sequences, are utilized in the construction of altered antibodies, preferably humanized antibodies, of this invention, by the following process. The same or similar techniques may also be employed to generate other embodiments of this invention.
- 20 A hybridoma producing a selected donor mAb is conventionally cloned, and the DNA of its heavy and light chain variable regions obtained by techniques known to one of skill in the art, e.g., the techniques described in Sambrook *et al.*, (Molecular Cloning (A Laboratory Manual), 2nd edition, Cold Spring Harbor Laboratory (1989)). The variable heavy and light regions
- 25 containing at least the CDR-encoding regions and those portions of the acceptor mAb light and/or heavy variable domain framework regions required in order to retain donor mAb binding specificity, as well as the remaining immunoglobulin-derived parts of the antibody chain derived from a human immunoglobulin are obtained using polynucleotide primers and reverse transcriptase. The CDR-encoding regions are identified using a known database and
- 30 by comparison to other antibodies.

A mouse/human chimeric antibody may then be prepared and assayed for binding ability. Such a chimeric antibody contains the entire non-human donor antibody V<sub>H</sub> and V<sub>L</sub> regions, in association with human Ig constant regions for both chains.

- 35 Homologous framework regions of a heavy chain variable region from a human antibody may be identified using computerized databases, e.g., KABAT®, and a human antibody having homology to the donor antibody will be selected as the acceptor antibody. A suitable light chain variable framework region can be designed in a similar manner.

A humanized antibody may be derived from the chimeric antibody, or preferably, made synthetically by inserting the donor mAb CDR-encoding regions from the heavy and light chains appropriately within the selected heavy and light chain framework. Alternatively, a humanized antibody can be made using standard mutagenesis techniques. Thus, the resulting humanized antibody contains human framework regions and donor mAb CDR-encoding regions. There may be subsequent manipulation of framework residues. The resulting humanized antibody can be expressed in recombinant host cells, e.g., COS, CHO or myeloma cells.

- 10 A conventional expression vector or recombinant plasmid is produced by placing these coding sequences for the antibody in operative association with conventional regulatory control sequences capable of controlling the replication and expression in, and/or secretion from, a host cell. Regulatory sequences include promoter sequences, e.g., CMV promoter, and signal sequences, which can be derived from other known antibodies. Similarly, a second expression vector can be produced having a DNA sequence which encodes a complementary antibody light or heavy chain. Preferably this second expression vector is identical to the first except insofar as the coding sequences and selectable markers are concerned, so to ensure as far as possible that each polypeptide chain is functionally expressed. Alternatively, the heavy and light chain coding sequences for the altered antibody may reside on a single vector.

25 A selected host cell is co-transfected by conventional techniques with both the first and second vectors (or simply transfected by a single vector) to create the transfected host cell of the invention comprising both the recombinant or synthetic light and heavy chains. The transfected cell is then cultured by conventional techniques to produce the engineered antibody of the invention. The humanized antibody which includes the association of both the recombinant heavy chain and/or light chain is screened from culture by appropriate assay, such as ELISA or RIA. Similar conventional techniques may be employed to construct other altered antibodies and molecules.

30 Suitable vectors for the cloning and subcloning steps employed in the methods and construction of the compositions of this invention may be selected by one of skill in the art. For example, the conventional pUC series of cloning vectors, may be used. One vector, pUC19, is commercially available from supply houses, such as Amersham (Buckinghamshire, United Kingdom) or Pharmacia (Uppsala, Sweden). Additionally, any vector which is capable of replicating readily, has an abundance of cloning sites and selectable genes (e.g., antibiotic resistance), and is easily manipulated may be used for cloning. Thus, the selection of the cloning vector is not a limiting factor in this invention.

Similarly, the vectors employed for expression of the antibodies may be selected by one of skill in the art from any conventional vector. The vectors also contain selected regulatory sequences (such as CMV promoters) which direct the replication and expression of heterologous DNA sequences in selected host cells. These vectors contain the above described DNA sequences which code for the antibody or altered immunoglobulin coding region. In addition, the vectors may incorporate the selected immunoglobulin sequences modified by the insertion of desirable restriction sites for ready manipulation.

The expression vectors may also be characterized by genes suitable for amplifying expression of the heterologous DNA sequences, e.g., the mammalian dihydrofolate reductase gene (DHFR). Other preferable vector sequences include a poly A signal sequence, such as from bovine growth hormone (BGH) and the betaglobin promoter sequence (betaglopro). The expression vectors useful herein may be synthesized by techniques well known to those skilled in this art.

The components of such vectors, e.g. replicons, selection genes, enhancers, promoters, signal sequences and the like, may be obtained from commercial or natural sources or synthesized by known procedures for use in directing the expression and/or secretion of the product of the recombinant DNA in a selected host. Other appropriate expression vectors of which numerous types are known in the art for mammalian, bacterial, insect, yeast, and fungal expression may also be selected for this purpose.

The present invention also encompasses a cell line transfected with a recombinant plasmid containing the coding sequences of the antibodies or altered immunoglobulin molecules thereof. Host cells useful for the cloning and other manipulations of these cloning vectors are also conventional. However, most desirably, cells from various strains of *E. coli* are used for replication of the cloning vectors and other steps in the construction of altered antibodies of this invention.

Suitable host cells or cell lines for the expression of the antibody of the invention are preferably mammalian cells such as NS0, Sp2/0, CHO, COS, a fibroblast cell (e.g., 3T3), and myeloma cells, and more preferably a CHO or a myeloma cell. Human cells may be used, thus enabling the molecule to be modified with human glycosylation patterns. Alternatively, other eukaryotic cell lines may be employed. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Sambrook *et al.*, cited above.

Bacterial cells may prove useful as host cells suitable for the expression of the recombinant Fabs of the present invention (see, e.g., Plückthun, A., Immunol. Rev., 130:151-188 (1992)).

However, due to the tendency of proteins expressed in bacterial cells to be in an unfolded or improperly folded form or in a non-glycosylated form, any recombinant Fab produced in a bacterial cell would have to be screened for retention of antigen binding ability. If the molecule expressed by the bacterial cell was produced in a properly folded form, that bacterial cell would be a desirable host. For example, various strains of *E. coli* used for expression are well-known as host cells in the field of biotechnology. Various strains of *B. subtilis*, *Streptomyces*, other bacilli and the like may also be employed in this method.

Where desired, strains of yeast cells known to those skilled in the art are also available as host cells, as well as insect cells, e.g. *Drosophila* and *Lepidoptera* and viral expression systems. See, e.g. Miller *et al.*, Genetic Engineering, 8:277-298, Plenum Press (1986) and references cited therein.

The general methods by which the vectors may be constructed, the transfection methods required to produce the host cells of the invention, and culture methods necessary to produce the altered antibody of the invention from such host cell are all conventional techniques. Likewise, once produced, the antibodies of the invention may be purified from the cell culture contents according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Such techniques are within the skill of the art and do not limit this invention. For example, preparation of altered antibodies are described in WO 99/58679 and WO 96/16990.

Yet another method of expression of the antibodies may utilize expression in a transgenic animal, such as described in U. S. Patent No. 4,873,316. This relates to an expression system using the animal's casein promoter which when transgenically incorporated into a mammal permits the female to produce the desired recombinant protein in its milk.

Once expressed by the desired method, the antibody is then examined for *in vitro* activity by use of an appropriate assay. Presently conventional ELISA assay formats are employed to assess qualitative and quantitative binding of the antibody to MAG. Additionally, other *in vitro* assays may also be used to verify neutralizing efficacy prior to subsequent human clinical studies performed to evaluate the persistence of the antibody in the body despite the usual clearance mechanisms.

The therapeutic agents of this invention may be administered as a prophylactic or post injury, or as otherwise needed. The dose and duration of treatment relates to the relative duration of the molecules of the present invention in the human circulation, and can be adjusted by one of skill in the art depending upon the condition being treated and the general health of the patient.

The mode of administration of the therapeutic agent of the invention may be any suitable route which delivers the agent to the host. The antagonists and antibodies, and pharmaceutical compositions of the invention are particularly useful for parenteral administration, i.e., subcutaneously, intramuscularly, intravenously, or intranasally.

Therapeutic agents of the invention may be prepared as pharmaceutical compositions containing an effective amount of the antagonist or antibody of the invention as an active ingredient in a pharmaceutically acceptable carrier. In the prophylactic agent of the invention, an aqueous suspension or solution containing the engineered antibody, preferably buffered at physiological pH, in a form ready for injection is preferred. The compositions for parenteral administration will commonly comprise a solution of the antagonist or antibody of the invention or a cocktail thereof dissolved in an pharmaceutically acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be employed, e.g., 0.9% saline, 0.3% glycine, and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well known sterilization techniques (e.g., filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the antagonist or antibody of the invention in such pharmaceutical formulation can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 mL sterile buffered water, and between about 1 ng to about 100 mg, e.g. about 50 ng to about 30 mg or more preferably, about 5 mg to about 25 mg, of an antagonist or antibody of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain about 250 ml of sterile Ringer's solution, and about 1 to about 30 and preferably 5 mg to about 25 mg of an engineered antibody of the invention. Actual methods for preparing parenterally administrable compositions are well known or will be apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania.

It is preferred that the therapeutic agent of the invention, when in a pharmaceutical preparation, be present in unit dose forms. The appropriate therapeutically effective dose can be determined readily by those of skill in the art. To effectively treat stroke and other neurological diseases in a human, one dose of up to 700 mg per 70 kg body weight of an antagonist or antibody of this invention should be administered parenterally, preferably *i.v.* or

i.m. (intramuscularly). Such dose may, if necessary, be repeated at appropriate time intervals selected as appropriate by a physician.

The antibodies described herein can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins and art-known lyophilization and reconstitution techniques can be employed.

In another aspect, the invention provides a pharmaceutical composition comprising anti-MAG antibody or a functional fragment thereof and a pharmaceutically acceptable carrier for use in the promotion of oligodendrocyte survival.

The following examples illustrate the invention.

#### **Example 1**

Anti-MAG monoclonal antibody, control mouse IgG1 and Tau 1 were purchased from Chemicon. A Western blot of rat tissues lysates, prepared under non-reducing conditions was purchased from Chemicon.

Cultures of primary oligodendrocytes were prepared from O<sub>2</sub>A progenitor cells isolated from P0-P2 Sprague-Dawley rat as described (Vinson et al, Mol. Cell Neurosci. Vol 22 2003) and differentiated in culture for 5 days at which point they extended out myelin-like membranes positive for myelin markers and expressed MAG at the cell surface (Vinson et al, Mol. Cell Neurosci. Vol 22 2003).

For survival assays, O<sub>2</sub>A cells were seeded in 48-well plates (5 x 10<sup>4</sup> per well) in 500 µl Sato's medium ( 400 ng/ml T3, 400 ng/ml T4, 2 mM glutamine, 50 U/ml penicillin and 50 µg/ml streptomycin, 5 ml N2 supplement (Life Technology) with 0.5% FCS and were used at 5 days in vitro. Glutamate and antibodies were diluted in cystine-free DMEM, and cystine in 1N HCl. All incubations were performed at 37°C for 16 hr. Cell survival was quantified by colorimetric MTT assay (Skaper et al 1990, P M Conn (Ed.), Methods in Neurosciences Vol. 2 p17-33, Academic Press). Absorbance was measured at 570 and 630 nm with a micro-ELISA spectrophotometer.

#### ***Immunohistochemistry***

Immunohistochemistry was conducted using standard procedures as described previously (Irving et al. 2001 Acta Neuropathol (Berl) 102, 627-35. Incubation with the primary antibody was conducted (Tau 1 1:500, Egr-1 1:200) overnight at 4°C. Using analySIS® imaging system



software, the number of *egr-1* positive nuclei were counted per 25µm<sup>2</sup> area. Twelve areas were measured from the contralateral and ipsilateral : striatum, hind limb cortex and cingulate cortex in two separate sections per animal (n=6/group). Operator was blinded to treatment groups.

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The anti-MAG antibody was utilised to examine the effect of engagement of cell surface MAG on the protection of oligodendrocytes from glutamate mediated cell death. O<sub>2</sub>A oligodendrocyte precursor cells were differentiated *in vitro* for 6 days. At this time, like adult rat brain, they expressed both long and short isoforms of MAG at the RNA level and stained positive for MAG at the cell surface and (Vinson et al, 2003). Treatment with 2 mM glutamate in cystine-free medium for 16 hours, reduced cell viability by 50% (Fig. 1). Death was not rescued by addition of NMDA receptor antagonist MK801 or AMPA receptor antagonist DNQX (data not shown) but was completely reversed by the addition of exogenous cystine (Fig. 1). Addition of anti-MAG antibody, but not control IgG reduced the degree of cell death, with maximum protection seen at 1 µg/ml (Fig. 1).

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WO 02/062383 demonstrates in a rat model of stroke that administration of an anti-MAG antibody results in an improvement in functional recovery which was accompanied by a marked reduction in lesion area measured seven days following transient MCAO. Animals treated with anti-MAG antibody showed reduced neuronal loss (approximately 40-50%) as detected by CFV staining and reduced damage to axonal tracts as delineated by Tau 1 immunostaining compared to IgG treated controls. Tau 1 immunostaining in oligodendrocytes is thought to be indicative of cellular integrity post-stroke (Irving et al, Acta Neuropathol. (Berl), 102, p627-35, 2001). Tau1 is increased in oligodendrocytes up to 24h following MCAO in the ischaemic hemisphere (Irving et al, J. Cereb. Blood Flow Metab. 17, p612-22, 1997, Valeriani et al, J. Cereb. Blood Flow Metab. 20, P765-771, 2000), but is lost as the white matter degenerates at 1 week following MCAO (Irving et al, Acta Neuropathol. (Berl) 102, p627-35, 2001). The number of Tau 1 positive, histologically normal, oligodendrocytes present within the ischaemic lesion was greater following anti-MAG antibody compared to Fig 2 without administration. The ability of the anti-MAG antibody to prevent Tau 1 loss in oligodendrocytes within the lesion area suggests that treatment may have maintained a degree of oligodendrocyte integrity.

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### **Example 2– Chimeric antibody**

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Seq ID No 7 provides the amino acid sequence of a chimeric immunoglobulin heavy chain in which the murine anti-MAG heavy chain variable region is associated with a functional immunoglobulin secretion signal sequence, and with an altered form of the human IgG1 constant region, in which Kabat residues 248 and 250 have been mutated to alanine in order to disable the effector functions of binding to FcγRI and complement protein C1q (Duncan,

A.R. and Winter, G. Localization of the C1q binding site on antibodies by surface scanning. Nature 332, 738-740, 1988. Duncan, A.R., Woolf, J.M., Partridge, L.J., Burton, D.R. and Winter, G. Localisation of the binding site for human FcR1 on IgG. Nature 332, 563-564, 1988). Such mutations are optionally made in order to customise the properties of an altered antibody to achieve a particular therapeutic effect – for example binding to and blocking the function of an antigen without activating lytic effector mechanisms.

MGWSCIIILFLVATATGVHSEIQLVQSGPELKKPGETNKISCKASGYTFTNYGMNWVKQAPGKGLKW  
 MGWINTYTGEPTYADDFTRFAFSLETSASTAYLQISNLKNEDTATYFCARNPINYYGINYEGYVM  
 DYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTF  
 PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELAG  
 APSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV  
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL  
 VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHN  
 HYTQKSLSLSPGK

Seq ID No 8 provides the amino acid sequence of a chimeric immunoglobulin light chain in which the murine anti-MAG light chain variable region is associated with a functional immunoglobulin secretion signal sequence, and with the human kappa constant region.

MGWSCIIILFLVATATGVHSNIMMTQSPSSSLAVSAGEKVTMSCKSSHSVLYSSNQKNYLAWYQQKPG  
 QSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTITNVHTEDLAVYYCHQYLSSLTFTGTGKLEIKR  
 TVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS  
 LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Similarly, the anti-MAG variable regions may be associated with immunoglobulin constant regions which lack mutations disabling effector functions. Seq ID No 9 the amino acid sequence of a chimeric immunoglobulin heavy chain in which the murine anti-MAG heavy chain variable region is associated with a functional immunoglobulin secretion signal sequence, and with a wild-type form of the human IgG1 constant region.

MGWSCIIILFLVATATGVHSEIQLVQSGPELKKPGETNKISCKASGYTFTNYGMNWVKQAPGKGLKW  
 MGWINTYTGEPTYADDFTRFAFSLETSASTAYLQISNLKNEDTATYFCARNPINYYGINYEGYVM  
 DYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTF  
 PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPPELLG  
 GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV  
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL  
 VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHN  
 HYTQKSLSLSPGK

From the information provided in Seq ID Nos 7, 8 and 9, cDNA inserts encoding these chimeric chains may be prepared by standard molecular biology techniques well known to those skilled in the art. Briefly, the genetic code is used to identify nucleotide codons encoding the desired amino acids, creating a virtual cDNA sequence encoding the chimeric protein. If the cDNA insert is desired to be expressed in a particular organism, then particularly favoured codons may be selected according to known codon usage biases. The desired nucleotide sequence is then synthesised by means of PCR amplification of a template comprising overlapping synthetic oligonucleotides which, as a contig, represent the desired sequence. The resulting product may also be modified by PCR or mutagenesis to attach restriction sites to facilitate cloning into a suitable plasmid for expression or further manipulations.

### **Example 3 - Chimeric antibody binds to rat MAG in ELISA**

Chimeric anti-MAG antibody containing the light and heavy chain CDRs of the invention was produced by transient transfection of CHO cells. Antibody concentration was determined by ELISA and estimated to be around 0.5 µg/ml. For MAG binding, commercially available ratMAG-Fc was used. Due to the fusion with human Fc bound chimeric antibodies could not be detected using anti-human IgG secondary antibodies. Instead, anti-human kappa light chain-specific antibody was used. Figure 3 shows that this chimeric antibody binds to MAG even at 1/64 dilution. An unrelated humanised antibody and culture supernatant from mock transfected cells did not generate any signal in this assay.

#### ***Procedure:***

ELISA microtiter plates (Nunc Maxisorp) were coated with 1 µg/ml rat MAG-Fc fusion protein (R&D systems; 538-MG) in PBS at 4°C overnight. Plates were washed twice with PBS and then blocked with PBS/BSA (1% w/v) for 1h at room temperature (RT). Culture supernatants from transiently transfected CHO cells were passed through 0.2µm filters and serially diluted in PBS/BSA starting at neat supernatant to 1/64 dilution. Sample dilutions were left at RT for 1h. Plates were then washed three times with PBS/Tween 20 (0.1%). Detection antibody was goat anti-human kappa light chain specific-peroxidase conjugate (Sigma A-7164) diluted at 1/2000 in PBS/BSA. The detection antibody was incubated for 1h at RT and the plates washed as above. Substrate solution (Sigma Fast OPD P-9187) was added and incubated until appropriate colour development was detected and then stopped using 3M H<sub>2</sub>SO<sub>4</sub>. Colour development was read at 490nm.

The application of which this description and claims forms part may be used as a basis for priority in respect of any subsequent application. The claims of such subsequent application

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may be directed to any novel feature or combination of features described herein. This may take the form of product, composition, process or use claims and may include, by way of example and without limitation, one or more of the following claims.

**CLAIMS**

1. A method of promoting oligodendrocyte survival in a human suffering or at risk of developing stroke or another neurological disease which comprises administering to said human a therapeutically effective amount of an anti-MAG antibody or a functional fragment thereof.
2. Use of an anti-MAG antibody or functional fragment thereof for the manufacture of a medicament for the promotion of oligodendrocyte survival in a human suffering from or at risk of developing stroke or another neurological disease.
3. A method according to claim 1 or use according to claim 2 wherein the anti-MAG antibody is an altered antibody.
4. A method according to claim 1 or a use according to claim 2 wherein the anti-MAG antibody is a chimeric antibody.
5. A method according to claim 1 or a use according to claim 2 wherein the anti-MAG antibody is a humanised antibody.
6. Use or a method according to claim 3 wherein the altered antibody or functional fragment thereof binds to MAG and comprises one or more of the following CDR's.

***Light chain CDRs***

<b><i>CDR</i></b>	<b><i>According to Kabat</i></b>
L1	KSSHSVLYSSNQKNYLA
L2	WASTRES
L3	HQYLSSLT

***Heavy chain CDRs***

<b><i>CDR</i></b>	<b><i>According to Kabat</i></b>
H1	NYGMN
H2	WINTYTGEPTYADDFTG
H3	NPINYYGINYEGYVMDY

7. Use or a method according to claim 6 wherein the altered antibody or functional fragment thereof comprises a heavy chain variable domain which comprises one or more CDR's selected from CDRH1, CDRH2 and CDRH3 and for a light chain variable domain which comprises one or more CDRs selected from CDRL1, CDRL2 and CDRL3 .
8. Use or a method according to claim 7 wherein the altered anti-MAG antibody or functional fragment thereof comprises:
  - a heavy chain variable domain ( $V_H$ ) which comprises in sequence hypervariable regions CDRH1, CDRH2 and CDRH3
  - and /or
  - a light chain variable domain ( $V_L$ ) which comprises in sequence hypervariable regions CDRL1, CDRL2 and CDRL3.
9. Use or a method according to claim 8 wherein the altered MAG antibody or functional fragment thereof comprises a heavy chain of Sequence ID No. 7 or 8 and/or a light chain Sequence ID No. 9.
10. Use or a method according to claim 8 wherein the altered anti-MAG antibody or functional fragment thereof comprises a heavy chain variable region selected from Sequence ID No. 10, 11, 12 or 13 and/or a light chain variable region selected from Sequence ID No. 14, 15, 16 or 17.
11. Use or a method according to claim 10 wherein the altered anti-MAG antibody or functional fragment thereof comprises a heavy chain variable region Sequence ID No. 10 and a light chain variable region selected from Sequence ID No. 14, 15, 16 or 17.
12. Use or a method according to claim 11 wherein the altered anti-MAG antibody or functional fragment thereof comprises a heavy chain variable region Sequence ID No. 10 and a light chain variable region selected from Sequence ID No. 14, 15, 16 or 17.

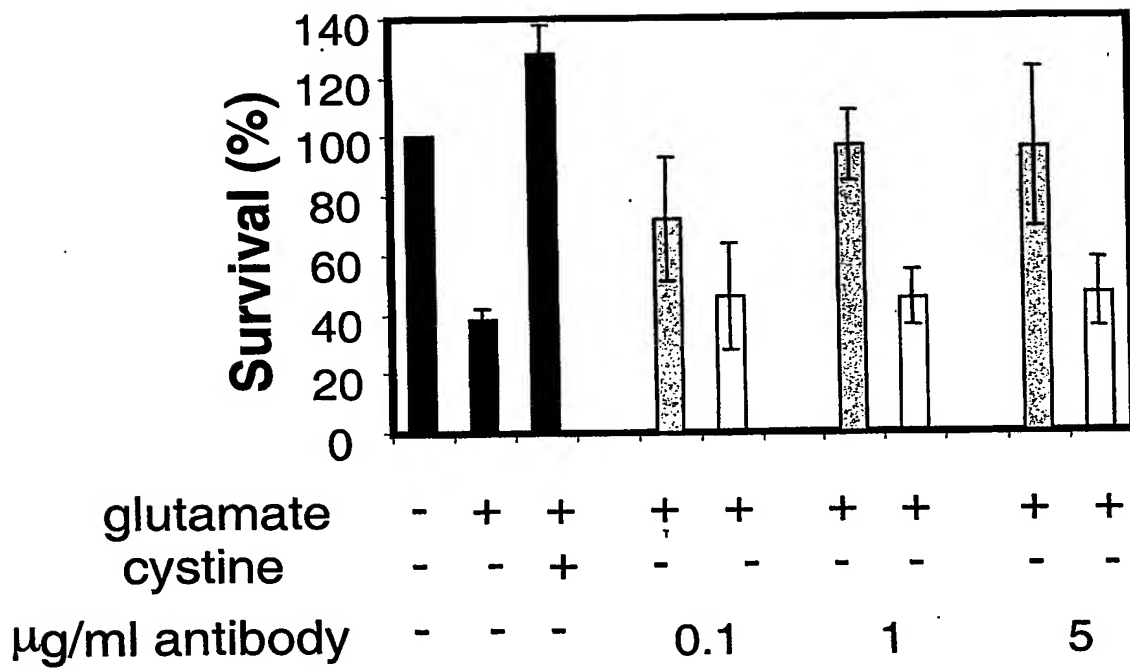
13. Use or a method according to claim 12 wherein the altered anti-MAG antibody or functional fragment thereof comprises a heavy chain variable region Sequence ID No. 10 and a light chain variable region selected from Sequence ID No. 14, 15, 16 or 17.
- 5 14. Use or a method according to claim 13 wherein the altered anti-MAG antibody or functional fragment thereof comprises a heavy chain variable region Sequence ID No. 10 and a light chain variable region selected from Sequence ID No. 14, 15, 16 or 17.

**Abstract**

A method of promoting oligodendrocyte survival in a human suffering or at risk of developing stroke or another neurological disease which comprises administering to said human a therapeutically effective amount of an anti-MAG antibody or a functional fragment thereof.



Figure 1



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Figure 2

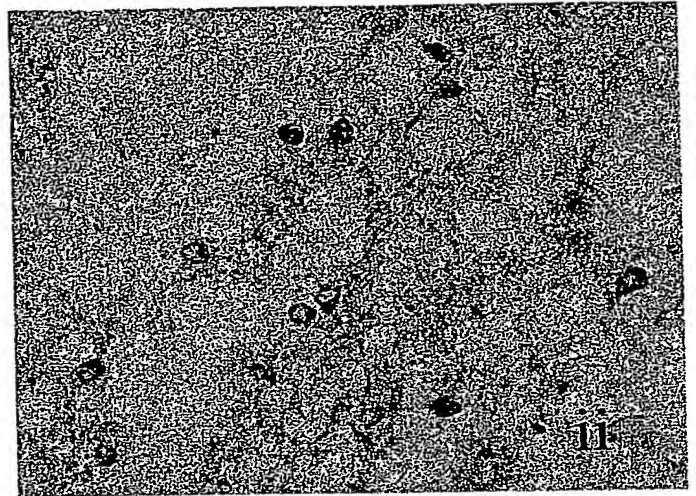
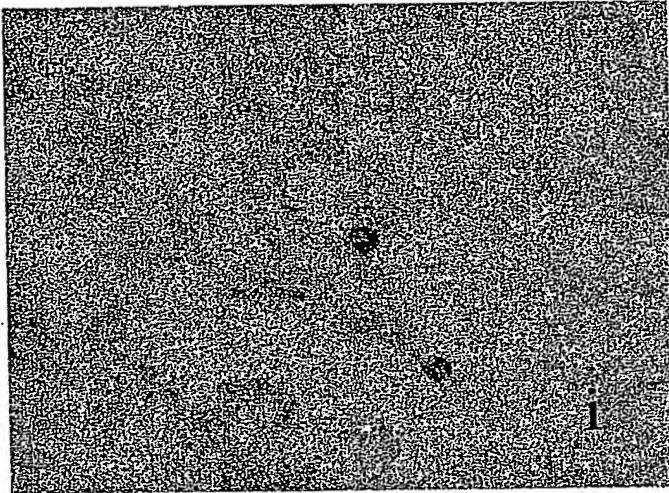
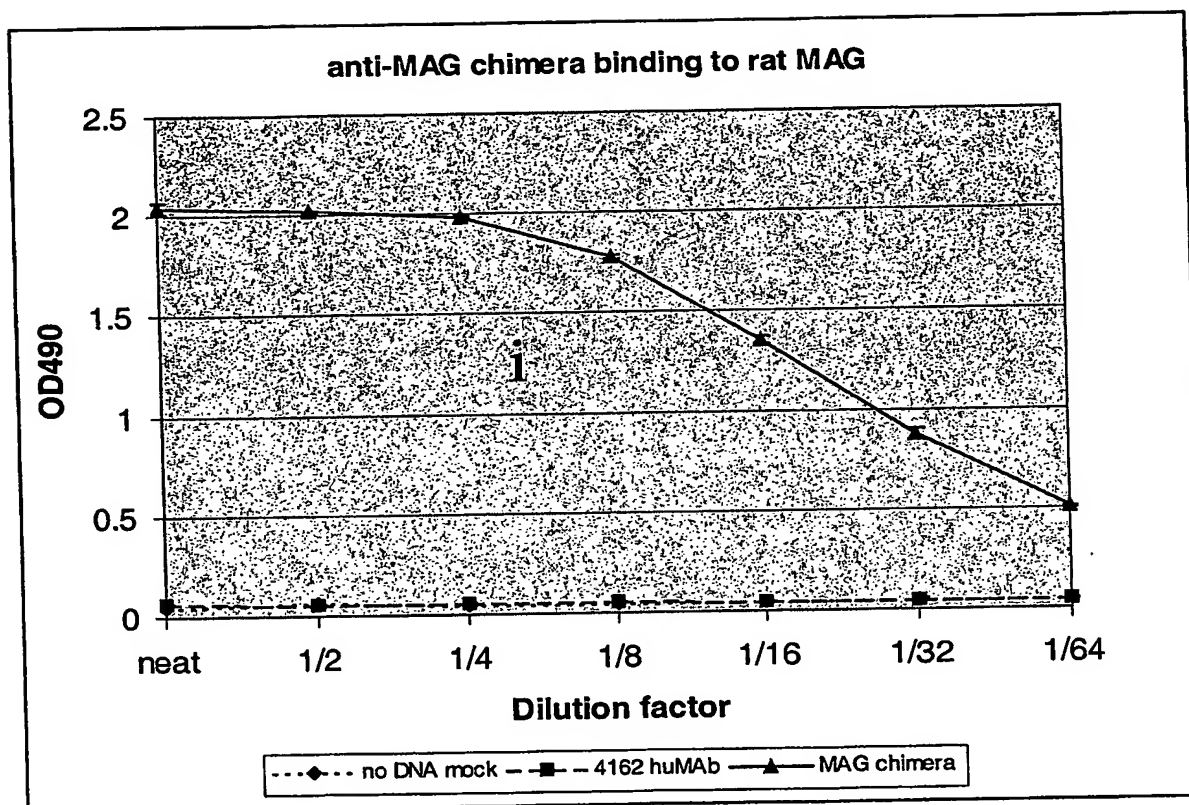


Figure 3



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